Laboratory Investigation

An Ex Vivo Model for the Reperfusion of Explanted Human Hearts

Kamuran A. Kadipasaoglu, PhD
Ger W. Bennink, MD
Jeff L. Conger, BS
Sinisa Birovljev, MD
Michele Sartori, MD
Fred J. Clubb, Jr., DVM, PhD
Hiroyuki Noda, MD
James J. Ferguson, MD
O.H. Frazier, MD, FACS

A model of an ex vivo-reperfused human heart was developed by using a modified Langendorff coronary perfusion circuit. The technical and physiologic aspects of re-establishing myocardial contractility are described. Preliminary studies were conducted in animals. In the present study, we obtained 12 human hearts that had been arrested with cardioplegic solution and excised from cardiac transplant recipients. The perfusate contained type-specific human donor red blood cells in a lactated Ringer's solution containing 5% dextrose. Myocardial contractility was successfully reestablished in 11 hearts and sustained for an average of 98 minutes (range, 79 to 180 minutes) at a coronary perfusion pressure of 80 mmHg. Left ventricular contraction pressures reached 40 mmHg (against intraventricular balloons at an internal pressure of 50 to 75 mmHg). Partial oxygen pressure (PO_2) dropped significantly across the empty beating myocardium (from 498 ± 40 mmHg to 219 ± 53 mmHg [mean ± SD]), but no significant change in hemoglobin saturation was observed. Myocardial failure generally stemmed from edematous changes leading to progressive impairment of myocardial relaxation. The intracoronary insertion of over-the-wire catheters did not adversely affect myocardial function.

In conclusion, an ex vivo-supported human heart model has been developed that may have a number of applications, including the preclinical evaluation of new interventional diagnostic and therapeutic techniques aimed at the coronary circulation, and the investigation of myocardial mechanics, preservation, and metabolism. ***(Texas Heart Institute Journal 1993;20:33-9)***

Key words: *Heart transplantation; heart catheterization; myocardial reperfusion, ex vivo; myocardium; oxygen consumption; resuscitation, myocardial*

From: *Cullen Cardiovascular Surgical Research Laboratories (KAK, GWB, JLC, MS, and HN), Cardiac Transplantation Program (SB and OHF), Cardiology Research (JJF), and Experimental Cardiovascular Pathology (FJC), Texas Heart Institute, Houston, Texas*

This work was partially supported by the Meadows Foundation, Grant No. 88120248; the Texas Higher Education Coordination Board, ATP Grant No. 2064; and a grant from Trimedyne, Inc., Tustin, California.

Address for reprints: *Kamuran A. Kadipasaoglu, PhD, Texas Heart Institute, P.O. Box 20345, Houston, TX 77225-0345*

Langendorff[1] first isolated and perfused a mammalian heart in 1895. Since then, researchers have used various animal resuscitation models to study the physiology and mechanics of cardiac function;[2] however, the use of a freshly excised human heart in a Langendorff perfusion circuit has not been reported.

An ex vivo-supported human heart model offers numerous potential benefits. Because the native anatomy of the human heart and the presence of pathologic lesions can provide a representation of coronary artery disease in humans, the ex vivo human heart model enables more precise determinations of the safety and the procedural efficacy of various interventional techniques compared with in vivo animal studies. Further, because the heart is actually beating, an ex vivo human heart model is better than cadaveric experimental models for assessing the preclinical performance of catheter-based interventional techniques on living tissue. In addition, the model allows immediate assessment of procedural effects at the histologic level. Finally, this model may provide a better understanding of the physiology and biochemistry of the cardiac muscle.

After conducting preliminary studies in animals, we developed a Langendorff-type model for the ex vivo reperfusion of human hearts harvested during cardiac transplantation from patients with end-stage cardiac disease. The technical and physiologic aspects of reestablishing myocardial contractility are described here.

Materials and Methods

Between November 1989 and May 1991, 12 hearts from patients (mean age, 46 years; range, 23 to 60 years) undergoing transplantation were available for this research. Of the hearts obtained, 8 were from men and 4 from women (Table I).

All procedures were approved by the Institutional Review Board at St. Luke's Episcopal Hospital.

Cardiac Excision and Coronary Perfusion Circuit

Cardiectomy was performed in 12 human cardiac transplant recipients at the mid-atrial level as described previously.[3] The myocardial mass was measured, and the patient's most recent catheterization diagram was used to locate coronary artery stenoses. All the explanted hearts were maintained at 4 °C until normothermic perfusion could be initiated; ischemic time did not exceed 90 minutes.

A modified Langendorff-type coronary perfusion circuit was used in all reperfusion procedures. The circuit consisted of a Model RV-500-1 PVC venous return reservoir (SciMed Life Systems, Inc.; Minneapolis, Minnesota, USA); a centrifugal pump (Biomedicus Medtronic, Inc.; Eden Prairie, Minnesota, USA) or roller pump (Sarns, Inc.; Ann Arbor, Michigan, USA); a Capiox 08 pediatric membrane oxygenator (Terumo Corp.; Tokyo, Japan); and a custom-designed compliance chamber for partial correction of cyclic fluctuations in pump pressure (Fig. 1). The perfusion circuit was primed with perfusate that contained heparin (10 IU/cc), glucose (500 g/L), insulin (10 IU/L), potassium (100 mEq/L),[4] 1 unit of group-specific packed human red blood cells (without functional platelets), 500 cc lactated Ringer's solution, and 5% (wt/vol) dextrose. The acid-base balance of the perfusate was corrected initially with 8.4% sodium bicarbonate, and the balance was monitored continuously during reperfusion. The gas (O_2, 95%; CO_2, 5%) flow rate was set at 1 L/min, and the perfusate temperature was maintained at 37 °C (Table II).

The hearts were cannulated either from the aorta (n = 4) with an interposing woven Cooley Dacron graft (Meadox Medicals; Oakland, New Jersey, USA) or from the ostia (n = 8) with 2 guiding catheters (9-F Giant Lumen Safetip GC, Interventional Medical;

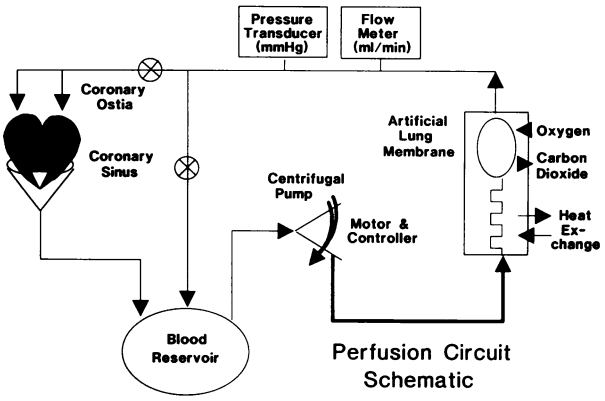


Fig. 1 *Schematic diagram of the modified Langendorff-type perfusion circuit.*

TABLE I. Patient Demographics

Patient No.	Sex/ Age (yr)	Reason for Transplant
1	M/50	Ischemic CMP
2	M/57	Ischemic CMP
3	M/43	Ischemic CMP
4	F/34	Viral CMP
5	M/33	ESCD/IHSS
6	M/49	Hypertrophic CMP
7	F/60	Idiopathic CMP
8	M/58	Ischemic CMP
9	M/58	Ischemic CMP
10	F/29	ESCD/Drug toxicity
11	F/52	Ischemic CMP
12	M/23	Congestive CMP

CMP = cardiomyopathy; ESCD = end-stage cardiac disease; IHSS = idiopathic hypertrophic subaortic stenosis

TABLE II. Prepump Perfusate* Characteristics

Perfusate Components	Numeric Results
O_2 (L/min)	1-1.5
Glucose (g/L)	500
Heparin (IU/cc)	10
Insulin (IU/L)	10
Temperature (°C)	35-38
Hct (%)	15-20
K^+ (mEq/L)	100
pH	7.3-7.4
HCO_3 (mEq/L)	15-25
Base excess (mEq/L)	0
PO_2 (mmHg)	>450
PCO_2 (mmHg)	<50

* Type-specific blood diluted with lactated Ringer's solution, 5% dextrose (1:1 vol/vol)

Danvers, Massachusetts, USA) attached to the circuit with a Y-shaped connector (Fig. 2). Electrocardiographic leads were placed on the aorta and on the right and left epicardial surfaces of the ventricles. Endotracheal tubes (9-mm ID, Mallinckrodt Critical Care; Glens Falls, New York, USA) were placed in the right and left ventricles and were filled with saline sufficient to contact the maximal surface area without stretching the ventricular wall. Right and left ventricular intraballoon pressures were 50 to 75 mmHg, as measured with disposable fluid-filled pressure transducers (Abbott Critical Care Systems; Chicago, Illinois, USA) that were connected to an 8-channel strip chart recorder (Series 3,000, Gould Instruments, Inc.; Cleveland, Ohio, USA).

Coronary Perfusion

Cardioplegia and hypothermia were reversed by starting normothermic coronary perfusion at 80 mmHg. Coronary sinus effluent was collected in a funnel directly below the heart and drained to the venous reservoir under gravity. After regular myocardial contractility was established, pressures across the balloons, coronary perfusion pressures, pump flow, and the electrocardiograms were monitored continuously and recorded on a multichannel physiologic monitor/recorder (Model 5962A, Hewlett-Packard; Andover, Massachusetts, USA). The partial oxygen pressure of arterial and venous blood samples was measured, and the arterial and venous oxygen saturation and content were calculated, by using an automated blood gas analyzer (AVL; Roswell, Georgia, USA). Spontaneous increases in the perfusion pressure were corrected by reducing the pump flow in cases where the roller pump was used.

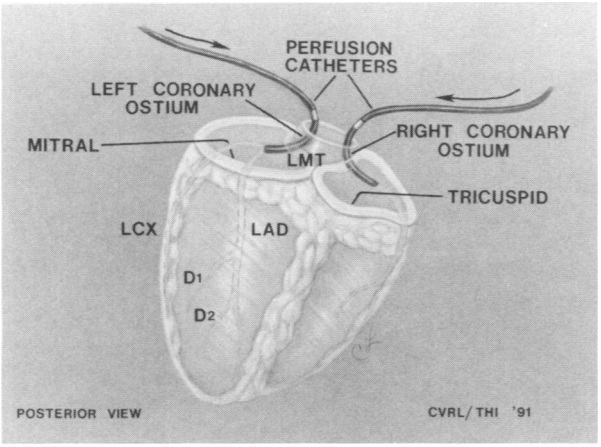


Fig. 2 *Schematic representation of the double ostial cannulation technique.*

D = diagonal arteries; LAD = left anterior descending artery; LCX = left circumflex artery; LMT = left main trunk

Once steady-state perfusion was established, over-the-wire transluminal catheters were introduced into the coronary arteries to assess their effect on myocardial function and perfusion variables.

To maintain reproducibility and higher data consistency, experiments were terminated if the coronary perfusion flow fell below 10 cc/min/100 g cardiac tissue or if the transballoon pressures (between the inside and the outside of the balloon) fell below 5 mmHg. After the experiment was terminated, the heart was disconnected from the circuit, weighed, and submerged overnight in 10% buffered formalin. Histologic and structural changes were assessed by using light and scanning electron microscopy.

Results

Of the 12 reperfused hearts, all but 1 (#3) started to beat and sustained regular ventricular contractions after routine supportive measures were administered. The period of ventricular contraction ranged from 79 minutes (in #1) to 180 minutes (in #4), with a mean of 96 ± 49 minutes (Table III). The mechanical and electrical activities of the human myocardium, together with the coronary perfusion flow and coronary perfusion pressure supplied by the roller pump are shown in Figure 3.

The starting value of the pump flow required to establish a perfusion pressure of 80 to 100 mmHg ranged from 100 to 1,150 cc/min (corresponding to

TABLE III. Perfusion Data from 11* Ex Vivo Human Hearts

Perfusion Variables	Numeric Results
Supported beating time (min)	96 ± 49
Arterial PO_2 (mmHg)	498 ± 40
Venous PO_2 (mmHg)	219 ± 53
Drop in oxygen saturation	Negligible
Circuit flow (range, cc/min)	100-1,150**
Heart rate (beats/minute)	15-69
Left ventricular pressure (transballoon, mmHg)	5-100

Beating time and PO_2 values are expressed as the mean ± SD. All other numbers are the absolute minimum and maximum values for each case averaged over 11 experiments. Range, therefore, does not indicate trends in individual experiments.

* The 12th heart failed to begin beating.
** Corresponds to 32 to 150 cc/min/100 g cardiac tissue.

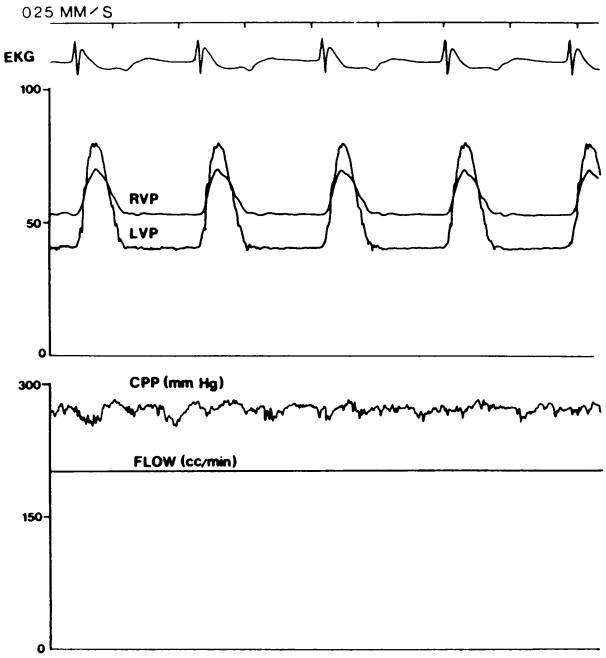


Fig. 3 *The mechanical and electrical activities of the ex vivo-supported human heart, along with the coronary perfusion flow and pressure supplied by the roller pump.*

CPP = coronary perfusion pressure; EKG = electrocardiogram; LVP = left ventricular pressure; RVP = right ventricular pressure

32 to 150 cc/min/100 g cardiac tissue). Subsequent adjustment of the roller pump output (especially for ischemic hearts) was necessary to maintain a constant coronary perfusion pressure, resulting in a gradual reduction of up to 60% of baseline flow over the course of the experiments. The mean partial oxygen pressures on the arterial and the venous sides were 498 ± 40 mmHg and 219 ± 53 mmHg, respectively, corresponding to a mean drop of 285 ± 59 mmHg across the myocardium. The oxygen saturation remained around 99% and did not show a detectable difference between the arterial and the venous sides. Regular electrical rhythm was established in each experiment. During contractions, maximal transballoon pressures were 20 and 40 mmHg for the right and left ventricles, respectively, in response to internal balloon pressures of 50 to 75 mmHg. All hearts tolerated the flow reduction caused by the transluminal introduction of diagnostic and therapeutic catheters. No rhythm disorders or reductions in the transballoon pressures were recorded; however, a transient decrease in heart rate was noted in some instances. During the coronary artery instrumentation period, the centrifugal pump flow decreased by approximately 30%; with the roller pump, the coronary perfusion pressure increased by 25% to 30%. These conditions were reversible on removal of the catheter.

Before perfusion, the coronary endothelium of the heart was examined under the scanning electron microscope and appeared normal (Fig. 4), with an intact membranous structure and tight gap junctions. After 2 hours of reperfusion, however, we detected lifting of the endothelial cells and focal blebs of the cell membranes (Fig. 5). Histologic examination before perfusion revealed typical myocardial architecture of a patient with cardiomyopathy (Fig. 6). After perfusion, regional changes were observed. The subepicardium had minimal changes, consisting of mild intercellular separation and intracytoplasmic vacuoles. In contrast, the subendocardium showed marked intercellular separation plus vacuolar and contraction-band changes within cardiac myocytes (Fig. 7).

Discussion

We have successfully used a modified Langendorff-type coronary perfusion system to resuscitate ex-

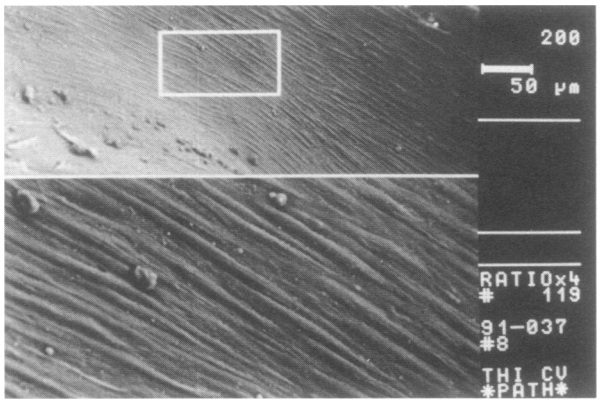


Fig. 4 *Scanning electron micrograph showing normal coronary endothelium of the human heart before perfusion.*

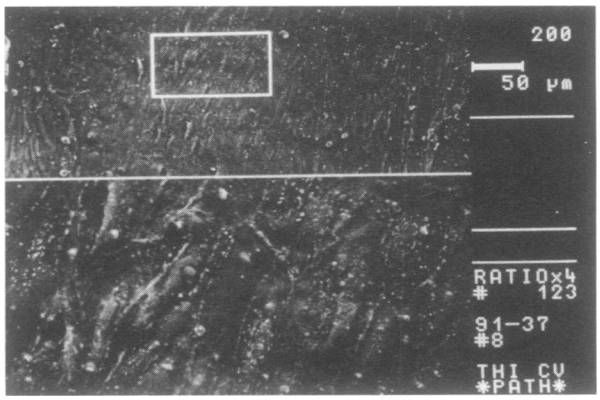


Fig. 5 *Scanning electron micrograph showing coronary endothelial cell damage after perfusion of the human heart. Boxed area highlights the junction of endothelium with platelets covering the region of endothelial cell loss.*

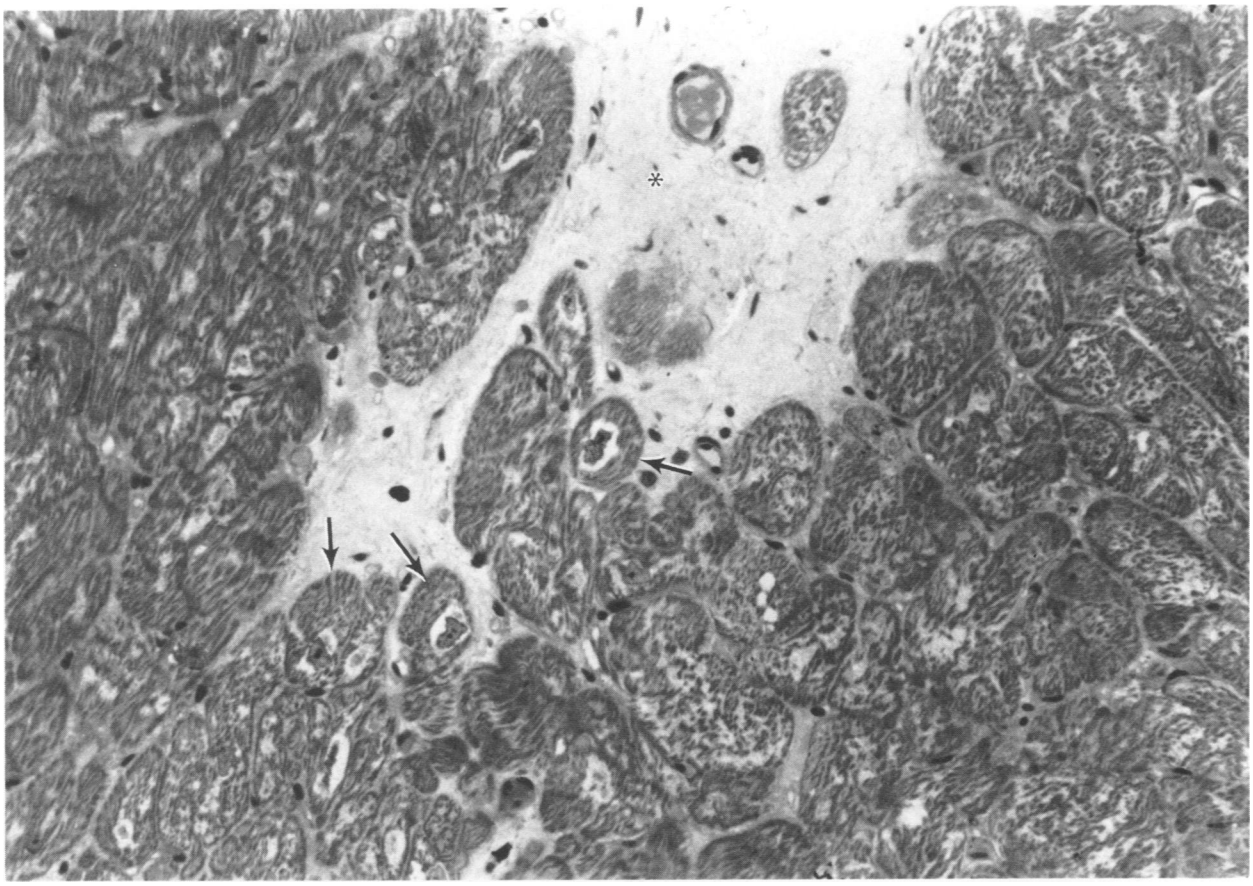


Fig. 6 *Subendocardium from explanted human heart with ischemic cardiomyopathy before perfusion. Fibrotic areas (asterisk) and vacuolated cardiac myocytes (arrows) are due to preexisting cardiomyopathy. (Glycolmethacrylate-embedded, 1-μm sections stained with H + E orig. x25)*

planted human myocardium for periods up to 3 hours. Mechanical contractility and electrical activity were maintained at levels within the predetermined experimental range during the period of ex vivo support. The empty beating hearts were able to extract oxygen, but myocardial oxygen demand did not reach levels high enough to dissociate the hemoglobin-bound oxygen. The sigmoid shape of the oxyhemoglobin saturation curve requires a greater change in PO_2 to reduce oxygen saturation than that observed in the present study. Two factors that may contribute to the lack of a significant change in oxygen saturation are the absence of left ventricular afterload in the empty beating heart model, and alternative energy production through anaerobic mechanisms in the myocardium. In future experiments it may be possible to extend the beating time by the addition of substrates such as oligomycin (an adenosine triphosphatase [ATPase] inhibitor);[5] adenosine (an adenosine triphosphate [ATP] precursor);[6] deoxyribose (which accelerates the de novo synthesis of the adenine nucleotides);[7] and L-glutamate (the precursor of a Krebs cycle intermediate that becomes depleted during anaerobic metabolism).[2] Monitoring lactate and adenylate levels in the future will also help assess the degree of metabolic contribution.

In our experiments with the roller pump, continuous adjustment of the pump flow rate was necessary to prevent the coronary perfusion pressure from exceeding a mean value of 80 mmHg. This persistent increase in the mean coronary perfusion pressure may have been caused by coronary vasoconstriction induced by either a decrease in the tissue's oxygen demand or an increase in edema within the deteriorating myocardium.

Edematous changes observed after reperfusion may have resulted from osmolar damage to the coronary endothelium. Eliminating excess water and metabolic end-products, possibly with a functional canine kidney or an ultrafiltration device, might have delayed the onset of edema and extended the functional viability of the beating heart.[8] In place of lactated Ringer's solution in the perfusate, we are currently evaluating the use of Krebs-Henseleit buffer,[9] which contains mannitol and raffinose,[10] to maximize our ability to control the chemical and osmolar balances in the perfusate.

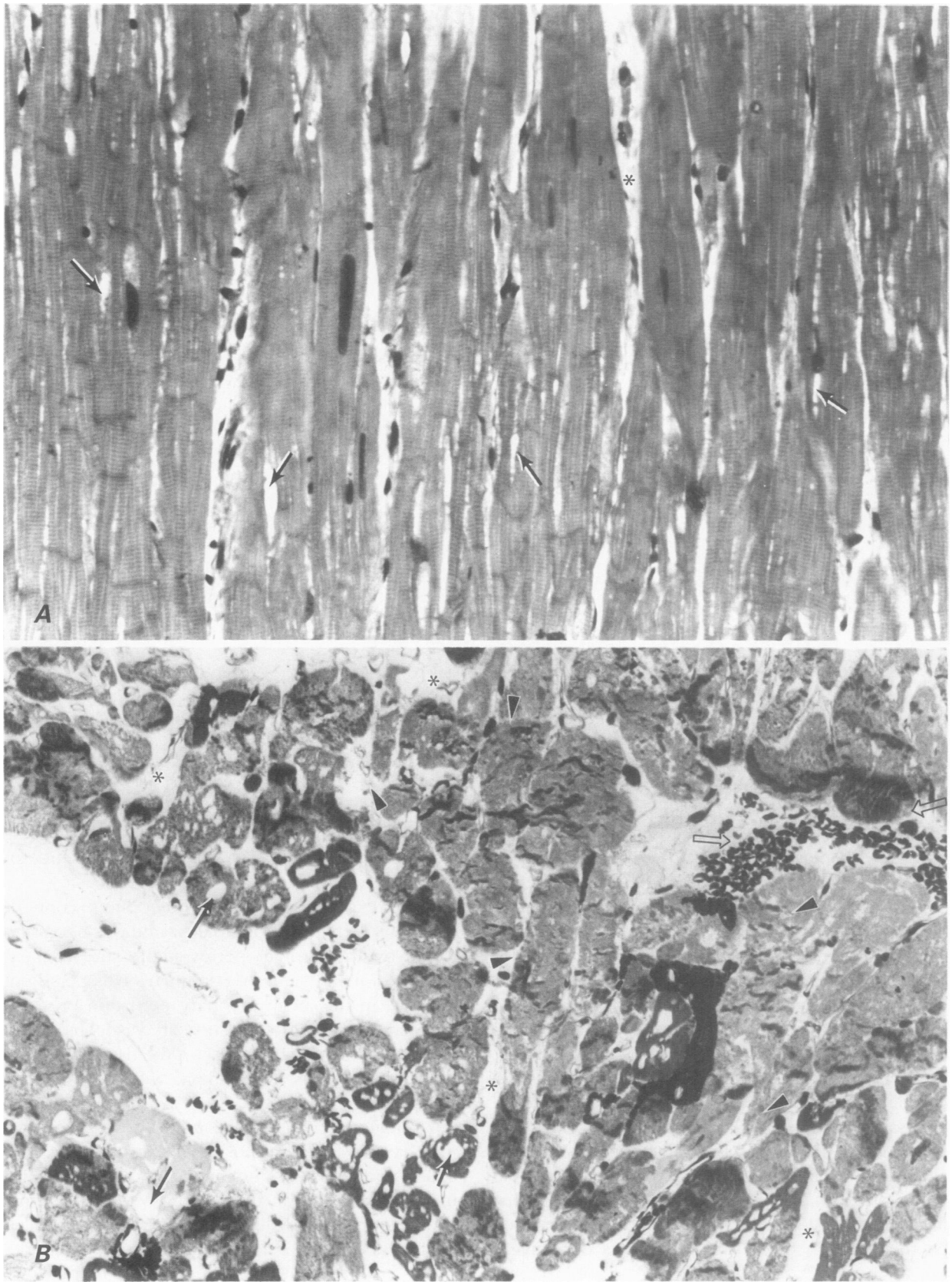


Fig. 7 ***A****) Subepicardium after perfusion, showing minimal cardiac myocyte alteration. Note intracytoplasmic vacuoles (arrows) and minor intracellular separation (asterisk).* ***B****) Subendocardium after perfusion, showing accentuated intracytoplasmic vacuoles (arrows), intercellular separation (asterisks), extravasated erythrocytes (open arrows), and myocytes with contraction-band changes (arrowheads). (Glycolmethacrylate-embedded, 1-μm sections stained with H + E orig. x25)*

This model presents a number of potential applications for the future. In interventional cardiology, it is an ideal apparatus for evaluating the preclinical performance of new diagnostic and therapeutic techniques, such as lasers, stents, atherectomy devices, and intravascular ultrasound. This model also offers a powerful tool for assessing myocardial preservation strategies, as well as for analyzing human ex vivo myocardial mechanics and metabolism. Developing these myriad applications will require much more work, but they will provide us with unique information that heretofore has been accessible only indirectly.

References

1. Langendorff O. Untersuchungen und Uberlebenden Saugethierhertzen. Pflugers Arc ges Physiol 1895;61:291.
2. Gharagozloo F, Melendez FJ, Hein RA, et al. The effect of amino acid L-glutamate on the extended preservation ex vivo of the heart for transplantation. Circulation 1987;76 (Suppl V):V-65-70.
3. Cooley DA. Techniques in cardiac surgery. Philadelphia: WB Saunders, 1984:90-1.
4. Gradinac S, Coleman GM, Taegtmeyer H, Sweeney MS, Frazier OH. Improved cardiac function glucose-insulin-potassium after aortocoronary bypass grafting. Ann Thorac Surg 1989;48:484-9.
5. Stringham JC, Southard JH, Anderson GM, Belzer FO. Mechanisms of ATP depletion in the cold-stored heart. Transplant Proc 1991;23:2437-8.
6. Demmy TL, Magovern JA, Kao RL, Magovern GJ. Resuscitation of injured myocardium with adenosine and biventricular assist. Ann Thorac Surg 1991;52:1044-51.
7. Pasque MK, Spray TL, Pellom GL, et al. Ribose-enhanced myocardial recovery following ischemia in the isolated working rat heart. J Thorac Cardiovasc Surg 1982;83:390-8.
8. Morimoto T, Golding LR, Stewart RW, et al. A simple method for extended heart-lung preservation by autoperfusion. ASAIO Trans 1984;30:320-4.
9. Nguyen VTB, Mossberg KA, Tewson TJ, Wong WH, Rowe RW, Coleman GM, Taegtmeyer H. Temporal analysis of myocardial glucose metabolism by 2-[^{18}F]fluoro-2-deoxy-D-glucose. Am J Physiol 1990;259:H1022-31.
10. Swanson DK, Pasaoglu I, Berkoff HA, Southard JA, Hegge JO. Improved heart preservation with UW preservation solution. J Heart Transplant 1988;7:456-67.